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South African Journal of Botany 72 (2006) 478 – 481
SOUTH AFRICAN JOURNAL OF BOTANY
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Short communication

# Cell fusion and cytomixis during microsporogenesis in *Brachiaria humidicola* (Poaceae)

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Received 1 November 2005; accepted 28 November 2005

**Abstract**

During cytological analysis of microsporogenesis in 28 polyploid accessions of *Brachiaria humidicola* (Poaceae) from the Embrapa Beef Cattle germplasm collection for breeding purposes, cell fusions were recorded in two accessions and chromosome transfer among meiocytes in one of them. Cell fusion between two to more than ten cells was recorded from prophase I to telophase II. In the syncyte, each nucleus maintained its integrity. In one of these accessions, cytomixis with characteristics never reported in any other plant species was recorded. It occurred among very small meiocytes that transferred the entire genome or part of it to normal meiocytes. Chromosome transfer occurred preferentially during telophase I and, during migration, chromatin showed structural alteration. Both abnormalities compromise pollen fertility. In the *Brachiaria* genus, polyploid accessions are, in general, apomictic, albeit pseudogamous. Consequently, fertile pollen is essential to fertilize the central nucleus of the embryo sac and ensure viable seeds production. Thus, accessions with high frequencies of meiotic abnormalities might be eliminated early from the breeding program.


*Keywords:* *Brachiaria humidicola*; Cell fusion; Cytomixis; Forage grass; Microsporogenesis

Meiosis is a continuous process involving several cytological events that result in the reduction of chromosome number by half, thus ensuring the constancy of ploidy in the species after fertilization. There is ample evidence that meiosis is controlled by a large number of genes (Gottschalk and Kaul, 1974; Baker et al., 1976; Golubovskaya, 1979, 1989). Disruption in any step of meiosis, due to environmental or genetic factors can affect gametic fertility. Depending on the severity of the abnormality, total sterility can be expected.

Cytological analyses performed on several *Brachiaria* species of the Embrapa Beef Cattle collection revealed a large amount of different meiotic (Mendes-Bonato et al., 2001a,b, 2002a,b, 2003; Risso-Pascotto et al., 2002, 2003a; Utsunomiya et al., 2004, 2005) and post-meiotic abnormalities (Junqueira Filho et al., 2003; Mendes-Bonato et al., 2004; Risso-Pascotto et al., 2005) which compromise pollen viability. In spite of the prevalent asexual reproduction by apospory of the *Panicum* type (Valle and Savidan, 1996; Araújo et al., 2000), these polyploid accessions in the genus *Brachiaria* are pseudogamous, i.e. fertile pollen grains are necessary to fertilize the two secondary nuclei of the embryo sac to ensure endosperm development (Alves et al., 2001).

*Brachiaria humidicola* is a species natural to Africa and widely used for pastures in the tropics, especially under poorly drained conditions. New cultivars are urgently needed to minimize the risk of extensive contiguous areas being planted to the only apomictic cultivar available commercially. New varieties are being sought to explore either the natural genetic variability among accessions or to generate novel genetic variability by intraspecific hybridization, since tetraploid sexual accessions have been identified (Valle and Glienke, 1991; Valle and Savidan, 1996). A new cultivar, cv Tupi, is scheduled to be released in Brazil in 2007 and was selected from the germplasm collection. There is an increase in interest in this species by breeders and producers thus justifying the effort to analyze the microsporogenesis of all the accessions of this species and related ones in the Embrapa Beef Cattle collection. During

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doi:10.1016/j.sajb.2005.11.004

cytological characterization of 28 accessions of *B. humidicola*, two showed cell fusion and one of these also cytomixis among meiocytes. These abnormalities are reported here.

Twenty eight of about 60 accessions of *B. humidicola* (Rendle) Schweick from the Embrapa Beef Cattle germplasm collection collected in wild East African savannas in the 1980s were analyzed cytologically. Site characteristics of accessions cultivated at the Embrapa Beef Cattle Research Center at Campo Grande, Mato Grosso do Sul, Brazil were: climate type Aw: tropical humid savanna; average annual precipitation = 1526 mm; average temperature = 22 °C; altitude 520 m; latitude = 20° 28′ S; longitude = 55° 40′ W; poor Dark Red Latossol soil composed of 59% sand; 8% silt and 33% clay; pH = 4.2.

Inflorescences for the meiotic study were collected in plots of 16 plants that represent each accession and fixed in a mixture of 95% ethanol, chloroform and propionic acid (6:3:2) for 24 h, transferred to 70% alcohol and stored under refrigeration until use. Microsporocytes were prepared by squashing and staining with 0.5% propionic carmine. Photomicrographs were taken in a Wild Leitz microscope using Kodak Imagelink — HQ, ISO 25 black and white film.

In addition to the expected meiotic abnormalities typical of polyploidy and affecting pollen viability, such as irregular chromosome segregation in the first and the second meiotic divisions, cell fusions were recorded in two accessions (H012, BRA004979 and H003, BRA004812) and chromosome transfer among meiocytes in one of them (H003, BRA004812). Cytological details during microsporogenesis classifies H012 as a hexaploid ($2n = 6x = 54$), with a basic chromosome number $x = 9$ and suggests that H003 is a heptaploid ($2n = 7x = 42$), with a basic chromosome number $x = 6$. Recent cytological analyses in *Brachiaria* revealed that the majority of species are polyploid (Penteado et al., 2000), derived from the predominant basic chromosome number in the genus $x = 9$, followed by $x = 7$ (Mendes-Bonato et al., 2002a; Utsunomiya et al., 2005). A new basic chromosome number $x = 6$ has recently been described in *B. dictyoneura* (Risso-Pascotto et al., 2006).

Table 1 shows the frequency of cells involved in these abnormalities. Cell fusion was detected among some meiocytes in several anthers. The fusions involved from 2 to more than 10 cells (Fig. 1). The majority of fused cells occurred in prophase I (Fig. 1a,b), but fusions were recorded until telophase II (Fig. 1e) giving rise to abnormal meiotic products (Fig. 1f). There was no nuclear fusion in the syncytes. Each genome maintained its integrity. Another interesting aspect observed in H003 was the difference in size of fused cells. Very small cells with an apparently normal genome were found fused with normal cells (Fig. 1c). Cell fusion had been reported in some *Brachiaria* species. In some *B. brizantha* genotypes, this phenomenon was restricted to male flowers of the raceme (Mendes-Bonato et al., 2001c); however, in accessions of other species they occurred in the hermaphrodite flowers (Mendes-Bonato et al., 2001a; Risso-Pascotto et al., 2003a,b; Utsunomiya et al., 2005). In 5 of 22 accessions of *B. jubata*, cell fusion was found to occur among two cells and, after normal cytokinesis, it produced eight normal microspores (Mendes-Bonato et al., 2003).

Table 1
Frequency of meiocytes affected by cell fusion and cytomixis

| Phase | No. of cells analyzed | | Cell fusion | | Cytomixis |
|---|---|---|---|---|---|
| | | | No. of affected cells | | No. of affected cells |
| | H003 | H012 | H003 | H012 | H003 |
| Zygotene | 200 | 541 | 100 | – | – |
| Pachytene | 200 | 322 | 14 | 54 | – |
| Diplotene | 200 | 122 | 6 | 1 | – |
| Diakinesis | 200 | 323 | 46 | 7 | – |
| Metaphase I | 200 | 221 | – | 17 | 27 |
| Anaphase I | 200 | 144 | 2 | 6 | 5 |
| Telophase I | 200 | 141 | 14 | 6 | 44 |
| Prophase II | 200 | 172 | – | 14 | – |
| Metaphase II | 200 | 176 | – | – | – |
| Anaphase II | 200 | 149 | – | – | 3 |
| Telophase II | 200 | 166 | 2 | – | – |
| Microspores | 200 | 302 | 5 | – | 21 |

Cell fusion was also reported in several other plant species (Nirmala and Rao, 1996), and may result from suppression of cell wall formation during premeiotic mitoses. In general, cell fusion leads to abnormal formation of pollen grains. In the present accessions that was also the case. According to Nirmala and Rao (1996), several factors may cause cell fusion such as exposure to chemicals, temperature, culture conditions, and genetic factors. Considering that the present accessions of *B. humidicola* were cultivated under similar environmental conditions, the results suggest genetic control of cell fusion.

Chromosome transfer among meiocytes of H003 was recorded in low frequencies, but showing a pattern never reported before in other plant species. The transfer of chromosomes always occurred between cells of different size. Normal microsporocytes received the entire genome, or part of it, from very small cells through large inter-cytomictic channels (Fig. 1g,h). In general, the genomes of the small cells involved showed chromosome stickiness. A similar process of structural alteration of migrating chromatin was also recorded in *B. nigropedata* (Utsunomiya et al., 2004). According to Feijó and Pais (1989) such agglutination eases the passage for migrating chromatin. Hyperploid cells involved in cytomixis were also observed in our accession (Fig. 1h). The result of cytomixis was the increase of the genome in the cells (Fig. 1i). The phenomenon was found to occur from metaphase I to microspore stage, but most frequently at telophase I. A normal meiocyte could receive chromosomes from two cells in different meiotic stages or one small cell could transfer part of its genome to two normal cells. The origin of the small cells involved in cytomixis is unclear. They were never observed alone inside the anthers.

Cytomixis is commonly reported in meiocytes especially during prophase I, when cytoplasmic channels exist among cells. Heslop-Harrison (1966a,b) demonstrated that cytoplasmic channels initiated in the preleptotene stage, persisted throughout the meiotic prophase and disappeared before meiosis II, when each meiocyte became totally isolated within the enclosing callose wall. Cytomixis has been reported to occur preferentially between genetically unbalanced types

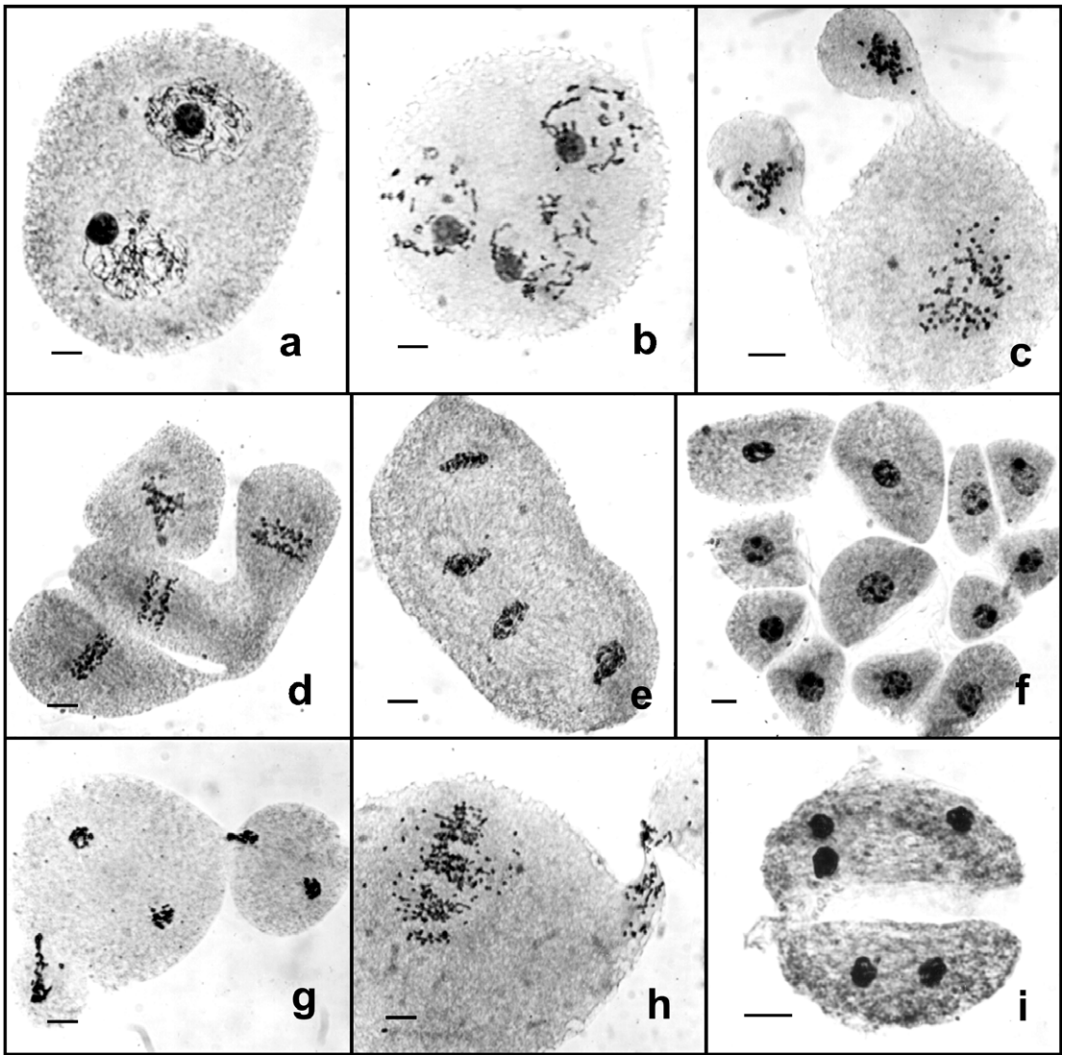


Fig. 1. Aspects of cell fusion and chromosome transfer among meiocytes: (a) fusion of two cells in pachytene; (b) fusion of three cells in diakinesis; (c) fusion of a normal cell and two small cells; (d) fusion of three cells in anaphase I; (e) fusion between two cells in telophase I; (f) abnormal meiotic products resulting from cell fusions; (g,h) chromosome transfer among meiocytes in telophase I (g) and metaphase (h). Observe that in (h) the normal cell is receiving chromosomes from the smallest cells, and that in (h) the receptive cell is hyperploid; (i) Telophase II with an extra nucleus resulting from cytomixis.

such as polyploids, hybrids, and apomictics (Gottschalk, 1970; Bahl and Tyagl, 1988). Perhaps the polyploid and the apomictic condition of this accession (Valle, unpublished data) predispose it to chromosome transfer among meiocytes. However, we cannot exclude the possibility of some genetic factor interfering with this phenomenon, because among the 25 accessions of *B. humidicola* analyzed, only this one was affected.

Despite the number of species in which cytomixis has been reported, its origin and significance are still unknown. Its role in the evolutionary process is contradictory, because it results in the formation of hyperploid and hypoploid cells, compromising pollen fertility. The influence of cytomixis on the generation of polyploid gametes can be expected in *Brachiaria*, a genus where polyploidy is predominant (Valle and Savidan, 1996; Penteado et al., 2000). However, when only a part of the genome is transferred, unbalanced and sterile gametes are formed. In the present accessions not only cell fusion and cytomixis contributed to pollen sterility, but also many other meiotic abnormalities typical of the polyploidy condition were recorded.

The *Brachiaria* breeding program depends on hybridization to produce novel genetic variability using sexual genotypes and the pollen of selected apomictic accessions or hybrids. Then, the hybrids that are produced are selected, among other traits, for good seed production in order for this technology to be widely adopted. Therefore accessions with high frequencies of meiotic abnormalities such as the ones observed for H003 and H012 present serious problems and should be eliminated early from the breeding program.

## References

Alves, E.R., Carneiro, V.T.C., Araújo, A.C.G., 2001. Direct evidence of pseudogamy in apomictic *Brachiaria brizantha* (Poaceae). Sexual Plant Reproduction 14, 207–212.

Araújo, A.C.G., Mukhambetzhanov, S., Pozzobon, M.T., Santana, E.F., Carneiro, V.T.C., 2000. Female gametophyte development in apomictic and sexual *Brachiaria brizantha* (Poaceae). Revue de Cytologie et Biologie Végétales — Le Botaniste 23, 13–26.

Bahl, J.R., Tyagl, B.R., 1988. Cytomixis in pollen mother cells of *Papaver dubium* L. Cytologia 53, 771–775.

Baker, B.S., Carpenter, A.T.C., Esposito, M.S., Esposito, R.E., Sandler, L., 1976. The genetic control of meiosis. Annual Review of Genetics 10, 53–134.

Feijó, J.A., Pais, M.S.S., 1989. Cytomixis in meiosis during the microsporogenesis in *Ophrys lutea*: an ultrastructural study. Caryologia 42, 37–48.

Golubovskaya, I.N., 1979. Genetic control of meiosis. International Review of Cytology 58, 247–290.

Golubovskaya, I.N., 1989. Meiosis in maize: *mei* genes and conception of genetic control of meiosis. Advances in Genetics 26, 149–192.

Gottschalk, W., Kaul, M.L.H., 1974. The genetic control of microsporogenesis in higher plants. The Nucleus 17, 133–166.

Gottschalk, W., 1970. Chromosome and nucleus migration during microsporogenesis of *Pisum sativum*. The Nucleus 13, 1–9.

Heslop-Harrison, J., 1966a. Cytoplasmic connections between angiosperm meiocytes. Annals of Botany 30, 221–230.

Heslop-Harrison, J., 1966b. Cytoplasmic continuities during spore formation of flowering plants. Endeavour 25, 67–72.

Junqueira Filho, R.G., Mendes-Bonato, A.B., Pagliarini, M.S., Bione, N.C.P., Valle, C.B., Penteado, M.I.O., 2003. Absence of microspore polarity, symmetric divisions and pollen cell fate in *Brachiaria decumbens* (Gramineae). Genome 46, 83–88.

Mendes-Bonato, A.B., Pagliarini, M.S., Silva, N., Valle, C.B., 2001a. Meiotic instability in invader plants of signal grass *Brachiaria decumbens* Stapf (Gramineae). Acta Scientiarum 23, 619–625.

Mendes-Bonato, A.B., Pagliarini, M.S., Valle, C.B., Penteado, M.I.O., 2001b. A severe case of chromosome stickiness in pollen mother cells of *Brachiaria brizantha* (Hochst) Staph (Gramineae). Cytologia 66, 287–291.

Mendes-Bonato, A.B., Pagliarini, M.S., Valle, C.B., Penteado, M.I.O., 2001c. Archesporial syncytes restricted to male flowers in a hexaploid accession of *Brachiaria brizantha* (Hochst) Stapf (Gramineae). The Nucleus 44, 137–140.

Mendes-Bonato, A.B., Pagliarini, M.S., Forli, F., Valle, C.B., Penteado, M.I.O., 2002a. Chromosome number and microsporogenesis in *Brachiaria brizantha* (Gramineae). Euphytica 125, 419–425.

Mendes-Bonato, A.B., Junqueira Filho, R.G., Pagliarini, M.S., Valle, C.B., Penteado, M.I.O., 2002b. Unusual cytological patterns of microsporogenesis in *Brachiaria decumbens*: abnormalities in spindle and defective cytokinesis causing precocious cellularization. Cell Biology International 26, 641–646.

Mendes-Bonato, A.B., Risso-Pascotto, C., Pagliarini, M.S., Valle, C.B., 2003. Normal microspore production after cell fusion in *Brachiaria jubata* (Gramineae). Genetics and Molecular Biology 26, 517–520.

Mendes-Bonato, A.B., Pagliarini, M.S., Risso-Pascotto, C., Valle, C.B., 2004. Abnormal pollen mitoses (PM I and PM II) in an interspecific hybrid from *Brachiaria ruziziensis* x *Brachiaria decumbens* (Gramineae). Journal of Genetics 83, 279–283.

Nirmala, A., Rao, P.N., 1996. Genesis of chromosome numerical mosaicism in higher plants. The Nucleus 39, 151–175.

Penteado, M.I.O., Santos, A.C.M., Rodrigues, I.F., Valle, C.B., Seixas, M.A.C., Esteves, A., 2000. Determinação de poliploidia e avaliação da quantidade de DNA total em diferentes espécies de gênero *Brachiaria*. Boletim de Pesquisa, vol. 11. Embrapa Gado de Corte, Campo Grande-MS, p. 19.

Risso-Pascotto, C., Pagliarini, M.S., Valle, C.B., 2002. Abnormal nucleolar cycle in microsporogenesis of *Brachiaria decumbens* (Gramineae). Cytologia 67, 355–360.

Risso-Pascotto, C., Pagliarini, M.S., Valle, C.B., Mendes-Bonato, A.B., 2003a. Chromosome number and microsporogenesis in pentaploid accession of *Brachiaria brizantha* (Gramineae). Plant Breeding 122, 136–140.

Risso-Pascotto, C., Pagliarini, M.S., Valle, C.B., 2003b. A mutation in the spindle checkpoint arresting meiosis in *Brachiaria ruziziensis*. Genome 46, 724–728.

Risso-Pascotto, C., Pagliarini, M.S., Valle, C.B., 2005. Symmetric pollen mitosis I and suppression of pollen mitosis II prevent pollen development in *Brachiaria jubata* (Gramineae). Brazilian Journal of Biological and Medical Research 38, 1603–1608.

Risso-Pascotto, C., Pagliarini, M.S., Valle, C.B., 2006. A new basic chromosome number for the genus *Brachiaria* (Trin.) Griseb. (Poaceae: Panicoideae: Paniceae). Genetic Research and Crop Evolution 53, 7–10.

Utsunomiya, K.S., Pagliarini, M.S., Valle, C.B., 2004. Chromosome transfer among meiocytes in *Brachiaria nigropedata* (Ficalho and Hiern) Stapf (Gramineae). Cytologia 69, 395–398.

Utsunomiya, K.S., Pagliarini, M.S., Valle, C.B., 2005. Microsporogenesis in tetraploid accessions of *Brachiaria nigropedata* (Ficalho and Hiern) Stapf (Gramineae). Biocell 29, 295–301.

Valle, C.B., Glienke, C., 1991. New sexual accessions in *Brachiaria*. Apomixis Newsletter 3, 11–13.

Valle, C.B., Savidan, Y., 1996. Genetics, cytogenetics, and reproductive biology of *Brachiaria*. In: Miles, J.W., Maass, B.L., Valle, C.B. (Eds.), Brachiaria: Biology, Agronomy, and Improvement Centro Internacional de Agricultura Tropical — CIAT/Empresa Brasileira de Pesquisa Agropecuária —CIAT, pp. 147–163.